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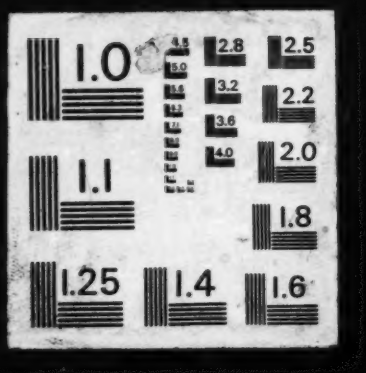

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Trans-Nonachlor Residues in Human Adipose Tissue

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trans-Nonachlor, chemically known as 1,2,3,4,5,6,7,8,8-nonachloro-3a,4,7,7a-tetrahydro-4,7-methanoindane, is a component of technical chlordane and technical heptachlor. According to Interpretation No. 23 of the Federal Insecticide, Fungicide and Rodenticide Act, as amended, technical chlordane is defined as containing 60% octachloro-4,7-methanotetrahydroindane and 40% related compounds. trans-Nonachlor is one of several chemicals found as a part of these related compounds and has been detected in technical heptachlor (Cochrane et al. 1970). Chlordane has been registered with the federal government since 1948 as an economic poison. It has been widely used as an insecticide and to a lesser extent, as a herbicide. Since its insecticidal efficacy includes pests of turf and lawns as well as household pests, its usage tends to bring this chemical in close proximity to humans.

The objective of this article is to report the discovery of trans-nonachlor in human adipose tissue and to provide a preliminary assessment of its geographic distribution in the United States. Although this report ascribes no clinical or symptomatic repercussions to these residues in humans, the finding of trans-nonachlor is indicative of exposure to chlordane and heptachlor. Other chemicals which are representative of chlordane and heptachlor exposure have also been reported in human tissues. Oxychlordane, a mammalian metabolite of several chlordane constituents, has previously been reported (Biros and Enos 1973). Heptachlor epoxide, derived from heptachlor or possibly in part from the heptachlor in technical chlordane, has also been reported in a national survey of human adipose tissue (Kutz 1974). trans-Nonachlor has also been reported in some environmental samples (Law and Goerlitz 1974, Lichtenstein 1971).

trans-Nonachlor was first detected as an unidentified component of adipose tissue extracts at the Pesticide Laboratory, Michigan Department of Public Health, which performs chemical analyses under contract for the National Human Monitoring Program (Kazen et al. 1974). These extracts were sent to the Environmental Toxicology Division, Research Triangle Park, N.C., for chemical characterization. When it was determined

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that the component was trans-nonachlor (Sovocool and Lewis 1975), a special study was organized under the National Human Monitoring Program to provide some preliminary data on the chemical epidemiology of this residue.

The National Human Monitoring Program functions to determine, on a national scale, the incidence and level of exposure to pesticides experienced by the general population and to identify changes and trends in these parameters when they occur. Further program information is detailed by Yobs (1971).

MATERIALS AND METHODS

Human adipose tissue was collected at various geographic locations throughout the conterminous United States. These tissues were extracted and analyzed for organochlorine pesticides using gas liquid chromatography with electron capture detection (GLC-EC). The presence of trans-nonachlor was confirmed, where possible, with gas liquid chromatography combined with mass spectrometry (GLC-MS). Residue levels of selected extracts were further estimated by multiple ion detection mass spectrometry (MID-MS).

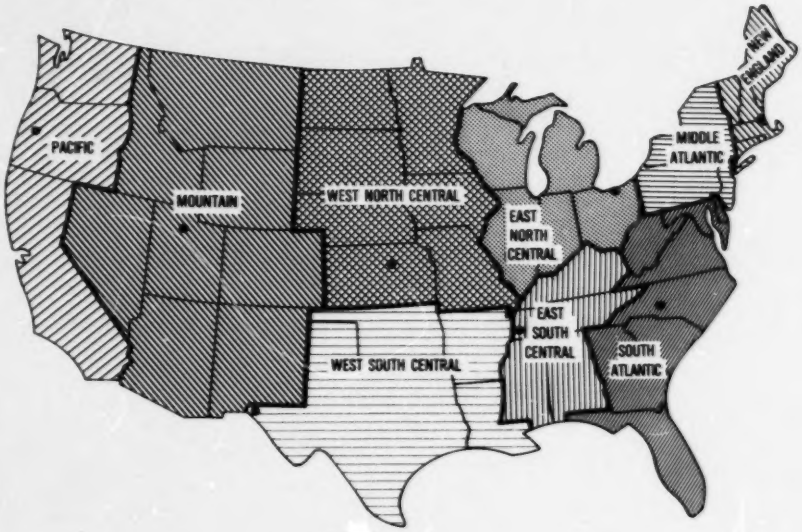

FIGURE 1

MAP OF THE UNITED STATES SHOWING THE LOCATION OF THE COLLECTION SITES IN THE 9 CENSUS DIVISONS

Five of the tissue specimens submitted from each of these sites during FY 1974 were extracted; these extracts were pooled to make a composite sample for each collecting site in each census division. The demographic (age, sex and race) description of the patients sampled to make up each composite are summarized in Table 1.

TABLE 1
Demographic Description of Patients Sampled
for trans-Nonachlor Analysis

Census Division	Geographic Location Sampled	No. of Patients Composited	Caucasian		Negro		Age Range	Samples Collected During
			Female	Male	Female	Male		
New England	Massachusetts	5	1	3	1	0	23-68	Postmortem
Middle Atlantic	New York	5	0	5	0	0	56-82	Postmortem
East North Central	Ohio	5	2	3	0	0	18-80	Postmortem
West South Central	Texas	5	4	1	0	0	10-65	Postmortem
South Atlantic	North Carolina	5	3	1	1	0	17-50	Postmortem
East South Central	Tennessee	5	4	1	0	0	25-77	Postmortem
West North Central	Kansas	5	3	2	0	0	41-67	Surgery
Mountain	Utah	5	2	2	1	0	22-73	Postmortem
Pacific	Oregon	5	4	1	0	0	29-43	Surgery

Sample Collection

Samples of human adipose tissue were obtained through cooperating medical pathologists and medical examiners at hospitals in cities selected according to a proportionate, stratified-random design. The conterminous 48 states were divided into 9 census divisions, according to the 1970 census of the United States. A city within each census division was selected from those already participating in the National Human Monitoring Program as the collection site for this special project (Figure 1).

Chemical Analysis

All tissues were extracted and cleaned-up according to a modified Mills-Olney-Gaither procedure (Thompson 1972) by laboratories under contract to the National Human Monitoring Program. Concentrated extracts, corresponding to the 6% diethyl ether in petroleum ether fraction from the florisil clean-up column were sent to the Environmental Toxicology Division for GLC-MS analysis.

Gas Chromatograph: Hewlett Packard 5700A with 1.8mm by 2mm i.d. X 4mm o.d. glass column packed with 1.5% OV-17, 1.95% OV-210 on 80-100 mesh Gas Chrom Q. The GC inlet temperature was 200°C. Helium flow rate was 42 ml/min. Temperature programmed runs were made from 80°C (2 min.) to 210°C at 8°C/min. Multiple ion detection runs were made isothermally at 190°C. The transfer line to the mass spectrometer was maintained at 210°C and the silicone membrane separator was kept at 180°C.

Mass Spectrometer: A Hewlett Packard 5930A quadrupole focusing mass spectrometer operated at 70eV electron energy and 400μ amps filament emission with target current of 310μ amps. MID runs used 100μ amps and high electron multiplier voltages. The ion source temperature was maintained at 180°C and the mass filter temperature at 110°C. Ions were detected using a Bendix continuous dynode electron multiplier with voltages varied between 1.5 and 2.5 Kv, as required for sufficient signal strength. Data were collected, stored and plotted using a Hewlett Packard 5932A Data System. Multiple ion detection was controlled by a suitable program provided by the manufacturer, and by the data system. The ions monitored were 405, 407, 409, 411 m/e. Data output also involved the use of a light beam oscillograph, as well as plotted mass spectra and reconstructed total ion chromatograms from the data system.

Using the periodic scanning mode of operation, with the Hewlett Packard 5932A Data System, mass spectra were generally scanned from 100 to 550 m/e at 160 m/e per sec., with scan cycle times of about 5.5 sec.

A standard sample of trans-nonachlor was secured from Velsicol Chemical Corporation, Chicago, Illinois. The melting point and mass spectrum of the standard were found to be in agreement with the literature (Damico et al. 1968 and Cochrane et al. 1970).

The technique of multiple ion detection (MID) was employed to estimate the trans-nonachlor levels in the samples from Massachusetts and Texas. Quantification by GC/MS was difficult to achieve, since no completely reliable method has yet been developed for trans-nonachlor.

Confirmation was based on the presence of the large M^+-Cl ion at 405 m/e (8Cl), and where possible, the much weaker molecular ion (M^+) at 440 m/e (9Cl). The weaker retro-Diels-Alder fragment ions were occasionally hidden under ions resulting from components in the adipose tissue. Gas liquid chromatographic retention times of standard trans-nonachlor were checked for all positive samples and were found to be identical to mass spectrum and GLC retention time of the compound identified in the human adipose tissue samples.

RESULTS AND DISCUSSION

The results of the analysis of human adipose tissue for trans-nonachlor are presented in Table 2. Of the nine composite samples analyzed, eight showed confirmable levels of trans-nonachlor. Estimates using the minimum detection limits indicated that levels in all positive samples were greater than 0.01 ppm. The MID estimates of residue levels in two samples are shown in parentheses.

TABLE 2

RESULTS OF ANALYSIS FOR TRANS-NONACHLOR IN HUMAN ADIPOSE COMPOSITE EXTRACTS

Census Division	GLC-Mass Spectrometry Confirmation
New England	Confirmed (0.04 ppm)[1]
Middle Atlantic	Confirmed
East North Central	Not Confirmed
West South Central	Confirmed (0.06 ppm)[1]
South Atlantic	Confirmed
East South Central	Confirmed
West North Central	Confirmed
Mountain	Confirmed at a lower level than others
Pacific	Confirmed

[1] Concentration estimated by multiple ion detection using the intensity of 409 m/e ion.

Positive samples were found both in widely diverse geographic areas as well as in widely diverse demographic distributions. The positive composites included samples of males and females, caucasians and negroes, different age groups and samples collected at both surgery and postmortem. Postmortem specimens were collected from hospital cases as well as from coroner/medical examiner sources. Therefore, residues were found in tissues received from patients having pathological conditions and from those killed by traumatic injuries.

One composite sample from Ohio did not contain confirmable quantities of trans-nonachlor. Although the composite sample from Utah contained confirmable quantities of trans-nonachlor, the level was lower than in the other positive samples. The exact explanation for the apparent absence of trans-nonachlor from the Ohio sample and for the low level in the Utah sample cannot be determined from this study. It is, however, important to note that this is a preliminary report containing data derived from a small subsample of the national human monitoring survey.

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U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
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Revised Chemicals Monitoring Guide for the National Pesticide Monitoring Program[1]

Milton S. Schechter[2]

A list of pesticides was promulgated several years ago in this Journal [*Pesticides Monit. J. 1*(1).20-21 (1967)] as a guide to Federal agencies participating in the National Pesticide Monitoring Program. The list contained chemicals believed to be of most interest because of their (1) extent and/or volume of usage and/or (2) degree of hazard to man, fish, and wildlife and/or (3) degree of persistence.

The Monitoring Panel of the Working Group on Pesticides responsible to the Council on Environmental Quality has suggested that the list be revised to take into account new pesticides which have been developed and changes in the use patterns of older pesticides. As mentioned in the previous article, these lists are suggested only as guides and are not to be considered as exclusive; all identifiable pesticide residues found in significant quantities in monitoring studies should be reported, including metabolic and/or breakdown products which are pesticidal or toxic.

In addition to the pesticides listed on the primary and secondary lists, there are some chemicals which, although not considered as pesticides per se, deserve special mention. One group of such chemicals (first identified as environmental pollutants in Sweden) are the PCB's or polychlorobiphenyls, which have been widely used as transformer oils, plasticizers, heat-exchange liquids, ink vehicles, etc. The PCB's have become spread throughout the environment and have been detected in fish and birds. When present in an environmental sample, the polychlorobiphenyls give rise to a complex series of peaks on gas chromatograms, and some of the peaks overlap or coincide with those given by some of the common organochlorine pesticides. The PCB's are relatively persistent toxic chemicals and, because of their adverse ecological effects, should be reported whenever detected in monitoring samples.

Another class of compounds which merits comment are the "dioxins." These are polychlorodibenzo-*p*-dioxins which have been found to occur as byproduct impurities in technical 2,4,5-T and pentachlorophenol. The dioxins are highly toxic chemicals which have been implicated in chick edema disease in chickens, in chloracne of industrial workers, and as teratogens in experimental animals. Because 2,4,5-T especially has been used on such a large scale, any of the dioxins detected in environmental samples should be reported, although little is known at present concerning the presence or persistence of dioxins in the environment. Current production of 2,4,5-T has been improved so that the level of tetrachlorodibenzo-*p*-dioxin is below 0.5 ppm in the technical product. In addition, many of the uses of 2,4,5-T have been suspended pending the outcome of hearings which are being held concerning the herbicide by the Environmental Protection Agency.

The problem of pollution of the environment by mercury is currently of tremendous interest. Instances of such pollution had been known in Japan ("Minimata disease" caused by eating fish contaminated with methyl mercury from industrial discharge into Minimata Bay and at Niigata) and in Sweden (primarily from paper mill slimicide treatments). Although mercury-containing pesticides (inorganic and organic) were listed on the initial

[1] This revised guide was drawn up after consultation with representatives from the U.S. Departments of Agriculture, Defense, the Interior, and Health, Education, and Welfare, and the Environmental Protection Agency, under the sponsorship of the Monitoring Panel of the Working Group.

[2] U.S. Department of Agriculture, Agricultural Research Service, Entomology Research Division, Pesticide Chemicals Research Branch, Agricultural Research Center, Beltsville, Md. 20705.

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Primary List of Chemicals for Monitoring, comparatively little monitoring for mercury in environmental samples had been done in the United States until the recent discovery of widespread pollution of certain rivers and lakes by this element (chiefly from electrolytic chlor-alkali plants), its conversion to methyl mercury by microorganisms, and the absorption or ingestion of the latter mercury compound by fish. It should be noted that such pollution does not seem to be due primarily to agricultural usage but instead is from industrial and natural sources.

Monitoring for mercury in the environment will no doubt continue to be carried out on a large scale until pollution by this toxic material and its compounds has been fully evaluated and brought under control, and the hazards to fish, wildlife, and man have been reduced to a tolerable level.

Problems of industrial pollution from chemicals which are not pesticides, such as PCB's and mercury, illustrate some of the complexities in the analysis of pesticide residues in environmental samples. Even more difficult is the interpretation of biological effects due to interactions of multiple pollutants and stresses on biological organisms. Clearly, a broader view of monitoring environmental samples must be taken than just a search for individual pesticide residues alone.

To reflect the increasing utilization of herbicides, a number of the important ones have been added, and atrazine has been moved from the secondary to the primary list because of its increased use. Some herbicides should be monitored on a specific crop or regional basis rather than countrywide, as for example fenac, which is a persistent, mobile herbicide used mainly on sugar cane, and propanil which is used mainly on rice.

Still important is the following statement quoted from the previous article that "Because of difficulties involved in screening samples for a multiplicity of pesticides and their important metabolites and degradation products, care should be used not only in the sampling and quantitative aspects of monitoring studies but especially in the identification aspects in order to assure reliability of reported results."

Primary list of chemicals for monitoring [1,2]

Common or Trade Name	Chemical Name
Aldrin	not less than 95% of 1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-*endo-exo*-5,8-dimethanonaphthalene
Amitrole	3-amino-*s*-triazole
Arsenic-containing pesticides (Inorganic and organic)	
Atrazine	2-chloro-4-(ethylamino)-6-(isopropylamino)-*s*-triazine
Azinphosmethyl (Guthion®)	*0,0*-dimethyl phosphorodithioate *S*-ester with 3-(mercaptomethyl)-1,2,3-benzotriazin-4(3*H*)-one
Benzene hexachloride (BHC)	1,2,3,4,5,6-hexachlorocyclohexane, consisting of several isomers and containing a specified percentage of *gamma* isomer [3]
Captan	*N*-[(trichloromethyl)thio]-4-cyclohexene-1,2-dicarboximide
Chlordane	at least 60% of 1,2,4,5,6,7,8,8-octachloro-3a,4,7,7a-tetrahydro-4,7-methanoindan and not over 40% of related compounds
2,4-D (including salts, esters, and other derivatives)	2,4-dichlorophenoxyacetic acid
DDT (including its isomers and dehydrochlorination products)	1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane; technical DDT consists of a mixture of the *p,p'*-isomer and the *o,p'*-isomer (in a ratio of about 3 or 4 to 1)
Dicamba	3,6-dichloro-*o*-anisic acid
Dieldrin	not less than 85% of 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo-exo*-5,8-dimethanonaphthalene
Dithiocarbamate pesticides: Maneb; Perbam; Zineb; etc.	[ethylenebis(dithiocarbamato)]manganese; tris(dimethyldithiocarbamato)iron; [ethylenebis(dithiocarbamato)]zinc;
Endrin	1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo-endo*-5,8-dimethanonaphthalene
Heptachlor	1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene
Heptachlor epoxide	1,4,5,6,7,8,8-heptachloro-2,3-epoxy-3a,4,7,7a-tetrahydro-4,7-methanoindan
Lindane	1,2,3,4,5,6-hexachlorocyclohexane, *gamma* isomer of not less than 99% purity
Malathion	diethyl mercaptosuccinate *S*-ester with *0,0*-dimethyl phosphorodithioate
Mercury-containing pesticides (inorganic and organic)	
Methoxychlor	1,1,1-trichloro-2,2-bis(*p*-methoxyphenyl)ethane; technical methoxychlor contains some *o,p'*-isomer also

Primary list of chemicals for monitoring [1,2]—*Continued*

Common or Trade Name	Chemical Name
Methyl parathion	*0,0*-dimethyl *0*-(*p*-nitrophenyl) phosphorothioate
Mirex	dodecachlorooctahydro-1,3,4-metheno-1*H*-cyclobuta[*cd*]pentalene
Nitralin (Planavin®)	4-(methylsulfonyl)-2,6-dinitro-*N*,*N*-dipropylaniline
Parathion	*0,0*-diethyl *0*-(*p*-nitrophenyl) phosphorothioate
PCNB	pentachloronitrobenzene
Picloram	4-amino-3,5,6-trichloropicolinic acid
Silvex (including salts, esters, and other derivatives)	2-(2,4,5-trichlorophenoxy)propionic acid
Strobane®	terpene polychlorinates containing 65% chlorine
2,4,5-T (including salts, esters, and other derivatives)	2,4,5-trichlorophenoxyacetic acid
TDE (DDD) (including its isomers and dehydrochlorination products)	1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane; technical TDE contains some *o,p'*-isomer also
Toxaphene	chlorinated camphene containing 67-69% chlorine
Trifluralin	α,α,α-trifluoro-2,6-dinitro-*N*,*N*-dipropyl-*p*-toluidine

Secondary list of chemicals for monitoring

Common or Trade Name	Chemical Name
DCNA (Botran®)	2,6-dichloro-4-nitroaniline
Carbaryl	1-naphthyl methylcarbamate
Demeton (Systox®)	mixture of *0,0*-diethyl *S*(and *0*)-[2-(ethylthio)ethyl] phosphorothioates
Diazinon	*0,0*-diethyl *0*-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate
Disulfoton (Di-Syston®)	*0,0*-diethyl *S*-[2-(ethylthio)ethyl] phosphorodithioate
Diuron	3-(3,4-dichlorophenyl)-1,1-dimethylurea
Endosulfan (Thiodan®)	1,4,5,6,7,7-hexachloro-5-norbornene-2,3-dimethanol cyclic sulfite
Fenac [4]	(2,3,6-trichlorophenyl)acetic acid
Fluometuron	1,1-dimethyl-3-(α,α,α-trifluoro-*m*-tolyl)urea
Inorganic bromide from bromine-containing pesticides	
Lead-containing pesticides such as lead arsenate	
Linuron	3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea
PCP	pentachlorophenol
Propanil [4]	3',4'-dichloropropionanilide
Triazine-type herbicides: [5]	
Simazine;	2-chloro-4,6-bis(ethylamino)-*s*-triazine;
Propazine;	2-chloro-4,6-bis(isopropylamino)-*s*-triazine;
Prometryne, etc.	2,4-bis(isopropylamino)-6-(methylthio)-*s*-triazine
TBA	2,3,6-trichlorobenzoic acid, usually available as mixed isomers

List of special chemicals for monitoring [6]

Common or Trade Name	Chemical Name
Polychlorobiphenyls (PCB's)	Mixtures of chlorinated biphenyl compounds having various percentages of chlorination
Polychlorodibenzo-*p*-dioxins	Dibenzo-*p*-dioxins having various degrees of chlorination such as the tetra-, hexa-, or octachlorodibenzo-*p*-dioxins, present as impurities in various chlorine-containing phenols and early samples of 2,4,5-T

[1] Chemical names are in accordance with *Chemical Abstracts*.
[2] An equal sign (=) at the end of a line signifies that the rest of the chemical name is to be joined without a space or hyphen at that point.
[3] Report individual isomers when possible.
[4] Some compounds are used primarily on one or two crops or in certain regions rather than countrywide; for example, the herbicides fenac and propanil are used mainly on sugar cane and rice, respectively.
[5] Note that atrazine has been moved to the Primary List.
[6] This list contains chemicals which, although not considered to be pesticides themselves, are of special interest in monitoring studies.

The author consulted the following Federal departments and individuals in revising the list of pesticides for monitoring:

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